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14. ABSTRACT

The overall objective of our synergistic team was to develop and apply a novel NMR-compatible model of human prostate cancer (PCa) to identify new hyperpolarized molecular imaging biomarkers for improved clinical management of PCa. We demonstrated that thin, precision-cut prostate tissue slice cultures (TSCs) could be maintained in an NMR-compatible bioreactor and that hyperpolarized ^{13}C spectroscopy could be employed to study real-time metabolism of normal and malignant tissues. The similarity of metabolism in TSCs compared to humans validates this new preclinical model and for the first time provides a representative experimental model that could be widely adopted by others to improve imaging modalities for prostate cancer. Our results suggest that hyperpolarized ^{13}C lactate may serve as a biomarker of prostate cancer.

15. SUBJECT TERMS

prostate cancer, imaging, tissue model

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INTRODUCTION

This project involves my lab at Stanford and the labs of Drs. Kurhanewicz and Ronen at UCSF. Overall, our goal is to identify hyperpolarized molecular imaging biomarkers for improved prostate cancer patient-specific treatment planning and early assessment of response to hormonal and chemotherapy. Specifically, my role is to supply prostate tissue cultures and develop optimal methods of culture. Our project has three aims: (1) to optimize and validate conditions for maintaining the structure and function of prostate tissue slice cultures (TCSs) in an nuclear magnetic resonance (NMR)-compatible, 3-dimensional tissue culture bioreactor, (2) to use the TSC/NMR bioreactor model to identify hyperpolarized metabolic biomarkers of prostate cancer presence and aggressiveness, and (3) to use the TSC/NMR bioreactor model to identify hyperpolarized metabolic biomarkers of response to hormonal and chemotherapy. In this final report, I summarize the contributions of my lab to this synergistic project.

BODY

The first Aim was to optimize and validate conditions for maintaining the structure and function of TSCs in an MRS-compatible, 3-D tissue culture bioreactor. As described in previous progress reports, this aim was achieved in year 1, with improvements in methodology in years 2 and 3. The use of TSCs for metabolic studies in a bioreactor is described in the recent joint publication of the Peehl and Kurhanewicz labs (Keshari et al., Prostate, in press, see "Reportable Outcomes" section below). The Peehl lab has also improved the longevity of TSCs in standard culture conditions, extending maintenance of structure and function up to 5 days (Maund et al., submitted to Lab. Invest., see "Reportable Outcomes" section below). A particularly noteworthy accomplishment of this latter study was the demonstration of maintenance of prostate cancer of different Gleason grades in TSCs, which has not been previously reported.

Aim 2 was to use the TSC/NMR bioreactor model to identify hyperpolarized metabolic biomarkers of prostate cancer presence and aggressiveness. As described in last year's progress report, the Peehl lab provided thin, precision-cut slices of prostate tissue to the Kurhanewicz lab and determined optimal protocols for transport to UCSF and culture. Using the bioreactor engineered by the UCSF investigators, robust and reproducible imaging signals were obtained. As described in the Prostate article by Keshari et al., the TSCs demonstrated steady state glycolytic and phospholipid metabolism, and bioenergetics, typical of prostate cancer in humans, validating the authenticity of the TSC model. Furthermore, the ^{13}C spectra following injection of hyperpolarized ^{13}C pyruvate into the bioreactor showed significantly increased pyruvate to lactate flux in prostate cancer as compared to benign prostate TSCs. The observed increase in flux in cancer correlated with increased expression of monocarboxylate transporters and lactate dehydrogenase activity. These findings suggest that hyperpolarized ^{13}C lactate may serve as a biomarker of prostate cancer, spurring further efforts for clinical translation of this technology.

Aim 3 was to identify hyperpolarized metabolic biomarkers of response to hormone and chemotherapy, As described in last year's progress report, we found that increasing the concentration of androgen (R1881) from 10 to 50 nM extended longevity in culture of the TSCs from normal tissues to 5 days. This year, we confirmed a similar effect on TSCs derived from malignant tissues of both high- and low-grade cancer. These findings are described in Maund et al. (submitted).

KEY RESEARCH ACCOMPLISHMENTS

- **Determined that our prostate-specific medium, PFMR-4A, maintains structure and function of normal and malignant prostate TSCs compatible with metabolic profiling**
- **Provided sufficient sets of normal and malignant TSCs to USCF to complete our ^{31}P spectroscopic analyses for publication**
- **Provided the UCSF team with tissue slice cultures of normal and malignant prostate from**

multiple donors

- **Evaluated several methods of transport of TSCs to UCSF and concluded that immediate transport to UCSF and overnight culture prior to imaging was optimal**
- **Cut tissue slices at 500-um instead of 300-um to improve ease of handling and to improve reproducibility of imaging signals**
- **Optimized several assays – LDH and Live-Dead – to improve quality control assessment of TSCs prior to imaging**
- **Determined that increasing the concentration of R1881 in the medium to 50 nM improved longevity of TSCs in culture and structural and functional maintenance of secretory differentiation**
- **Determined that increasing R1881 in the medium to 50 nM improved longevity of cancer of different Gleason grades in TSCs**
- **Demonstrated that TSCs provide an authentic and accurate model of the metabolism of the normal and malignant human prostate**

REPORTABLE OUTCOMES

Keshari, K.R., Sriram, R., van Criekinge, M., Wilson, D.M., Wang, Z.J., Vigneron, D.B., Peehl, D.M. and Kurhanewicz, J. Metabolic reprogramming and validation of hyperpolarized ^{13}C-lactate as a prostate cancer biomarker using a human prostate tissue slice culture bioreactor. Prostate, in press

Maund, S.L., Nolley, R. and Peehl, D.M. Optimization and comprehensive characterization of a faithful tissue culture model of the normal and malignant human prostate. Submitted to Lab Invest

CONCLUSIONS

The Peehl lab accomplished its goal of providing numerous, well-characterized TSCs of the normal and malignant human prostate to collaborators at UCSF. In conjunction with technologic development of a bioreactor and hyperpolarized imaging at UCSF, the Peehl lab improved culture methodology for TSCs that facilitated metabolic imaging studies at UCSF. Our results show that TSCs provide a realistic preclinical model of prostate cancer, perhaps prompting widespread adoption of this model by others for studies of metabolic imaging. The field remains in need of better methods for reliably imaging prostate cancer and predicting risk of aggressive disease. Our findings suggest that hyperpolarized ^{13}C lactate may serve as a biomarker of prostate cancer.

REFERENCES

None.

APPENDICES

None.